Leading Edge
Review
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Autoimmunity and the Clearance of Dead Cells

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To maintain organismal homeostasis, phagocytes engulf dead cells, which are recognized as dead by virtue of a characteristic "eat me" signal exposed on their surface. The dead cells are then transferred to lysosomes, where their cellular components are degraded for reuse. Inefficient engulfment of dead cells activates the immune system, causing disease such as systemic lupus erythematosus, and if the DNA of the dead cells is not properly degraded, the innate immune response becomes activated, leading to severe anemia and chronic arthritis. Here, we discuss how the endogenous components of dead cells activate the immune system through both extracellular and intracellular pathways.

Introduction

In the Japanese movie *Departures* (*Okuribito* in Japanese), which won the 2009 Oscar for best foreign language film, death is regarded as a gate. The deceased are gently washed, dressed, and placed in a coffin for departure into the next life. Similarly, when the cells in our bodies die, an elaborate process takes place to remove them and to give them a new life by using their components.

Many extra cells are generated and die during animal development. In human adults, billions of cells die every day as part of the body's natural processes. Cells that become damaged by microbial infection or mechanical stress also die. The cell death that occurs in the physiological setting is programmed, and is therefore called programmed cell death (Lockshin and Zakeri, 2001). Apoptosis is the major death process, but necrosis and autophagic cell death have also been proposed to play roles in programmed cell death (Kroemer et al., 2009). Dying cells secrete a "find me" signal, and they expose an "eat me" signal on their surface. In response to the "find me" signal, macrophages approach the dead cells; they then recognize the "eat me" signal (Ravichandran and Lorenz, 2007). Using sophisticated cell machinery, the phagocytes ingest the dead cells, direct them to lysosomes, and degrade their cellular components into basic biochemical building blocks: amino acids, nucleotides, fatty acids, and monosaccharides. These molecules will be released from the lysosomes and reused to make new macromolecules. In definitive erythropoiesis, the process by which red blood cells are generated, the nuclei are extruded from erythroid precursor cells at the final differentiation stage and are engulfed by macrophages (Chasis and Mohandas, 2008). The machinery used for the engulfment and degradation of the extruded nuclei appears similar to that used for the removal of apoptotic cells.

Mice deficient in the engulfment of apoptotic cells develop systemic lupus erythematosus (SLE)-type autoimmune diseases (Hanayama et al., 2004). A defect in the degradation of the chromosomal DNA from engulfed cells in mice activates macrophages, leading to lethal anemia in embryos and chronic arthritis in adults (Kawane et al., 2001; Kawane et al., 2006). These observations indicate that dead cells and the nuclei expelled from erythroid precursor cells need to be swiftly cleared for animals to maintain homeostasis.

Programmed Cell Death

Based on morphological and biochemical criteria, four different cell-death processes (apoptosis, cornification, necrosis, and autophagy) have been officially proposed (Kroemer et al., 2009). In apoptosis, the cell and nuclei condense and become fragmented and are engulfed by phagocytes (Kerr et al., 1972). Apoptosis is regulated by gene products, and programmed cell death has often been used synonymously with apoptosis. However, necrosis is also regulated by gene products (Cho et al., 2009; He et al., 2009), and it may be preferable to use the term programmed cell death in only its more general sense, that is, to refer to any cell-death process that is programmed into animal development.

It is unclear to what extent the other proposed forms of cell death can be classified as programmed cell death. Of them, autophagy, in which organelles and macromolecules are trapped by the cell's own membranes and degraded in its lysosomes, is a process by which cells survive in starvation conditions (Ohsumi, 2001). Autophagy has been proposed as a cell-death process because cells undergoing severe or prolonged autophagy may die, and dying cells often show a characteristic, autophagic morphology (Tsujimoto and Shimizu, 2005). However, there are no convincing data supporting the notion that autophagy kills the cells, and hence the term autophagic cell death may be misleading (Kroemer and Levine, 2008). Cornification, a cell-differentiation process, describes the cell death that occurs at the final step in the natural differentiation of skin cells (Lippens et al., 2005). Simi-

larly, the differentiation of the lens epithelial cells of the eye to fiber cells is accompanied by the degradation of nuclei, mitochondria, and endoplasmic reticulum (Bassnett, 2002), which can also be regarded as a cell-death process. However, it may not be appropriate to classify this cell-differentiation process as programmed cell death. In addition, although necrosis is mediated by gene products, it occurs only when apoptosis is blocked or when cells receive strong death signals under pathological conditions. Thus, we believe that apoptosis accounts for most of the physiological cell death during animal development and in the cell turnover that occurs daily.

Apoptosis

Apoptosis is activated by two pathways, the intrinsic and extrinsic pathways (Ow et al., 2008). In the intrinsic pathway, which operates in developmentally controlled and genotoxic agent-mediated apoptosis, BH3-only members of the Bcl-2 family are transcriptionally upregulated and stimulate the release of cytochrome C from the mitochondria. Together with Apaf-1, cytochrome C activates caspase 9, which leads to the activation of downstream caspases, including caspases 3 and 7. The antiapoptotic members of the Bcl-2 family inhibit the release of cytochrome C from mitochondria by a mechanism that has not been well elucidated. This intrinsic pathway is thought to be well conserved in metazoans, but its key step, the release of cytochrome C from mitochondria, is not observed in the nematode *C. elegans* or in the fruit fly *Drosophila* (Oberst et al., 2008).

Fas ligand (FasL), tumor necrosis factor (TNF), and TRAIL (TNF-related apoptosis-inducing ligand) are type II membrane proteins that can activate the extrinsic death pathway (Krammer, 2000; Nagata, 1997; Strasser et al., 2009). The binding of FasL to its receptor (Fas) induces the formation of the death-inducing signaling complex (DISC), consisting of Fas, an adaptor protein (FADD), and procaspase 8. Formation of the DISC leads to the processing and activation of caspase 8. Depending on the cell type, there are two pathways that can be activated downstream of caspase 8. In type I cells (for example, thymocytes), caspase 8 directly activates caspase 3 to kill the cells; in type II cells (hepatocytes), caspase 8 cleaves Bid, a BH3-only member of the Bcl-2 family, and the cleaved Bid (tBid) induces the release of cytochrome C from the mitochondria, which leads to the activation of the caspase 9-caspase 3 pathway.

In both the intrinsic and extrinsic pathways, apoptosis is completed by the cleavage of a set of cellular proteins (more than 500 substrates) by effector caspases (caspases 3 and 7) (Lüthi and Martin, 2007; Timmer and Salvesen, 2007) (http://cutdb.burnham.org/; http://bioinf.gen.tcd.ie/casbah/). The massive protein cleavage is probably responsible for the morphological and biochemical changes that occur during apoptosis, and for killing the cells. However, with a few exceptions (see below), the physiological meaning of the specific cleavage events is not clear. In addition to killing the cells, caspase activation is involved in the cells' production of the "find me" and "eat me" signals sensed by phagocytes.

Apoptotic DNA Degradation and Membrane Blebbing

One of notable hallmarks of apoptosis is DNA fragmentation, the cleavage of chromosomal DNA into 180 bp nucleosomal units (Wyllie, 1980). This process, accomplished by CAD (caspase-activated DNase), also called DFF-40 (DNA fragmentation factor 40), is the most representative example of how caspase activation causes a characteristic feature of apoptosis (Enari et al., 1998; Liu et al., 1997). In healthy cells, CAD is complexed with its inhibitor, ICAD (inhibitor of CAD), also called DFF-45 (Enari et al., 1998; Liu et al., 1997; Sakahira et al., 1998), which also acts as a chaperone for CAD to ensure its correct folding (Sakahira et al., 2000). Caspase 3 cleaves ICAD at two positions (Sakahira et al., 1998), which allows CAD to form a homodimer that has a scissor-like structure (Woo et al., 2004). CAD carries a nuclear-localization signal and cleaves DNA in the nucleus via specific histidine residues (Sakahira et al., 2001) located in the deep cleft between the "blades" of the "scissors." This structure prevents CAD from accessing the DNA on nucleosomes, but allows it access to DNA in the spacer regions between them, which explains why the chromosomal DNA is degraded into nucleosomal units during apoptotic cell death. CAD generates DNA fragments with a 3′-hydroxyl group. This group is identified by TUNEL (terminal transferase-mediated dUTP nick end labeling) staining, which is widely used to detect apoptotic cells in vitro and in vivo. At the early stage of apoptosis, DNA is degraded into relatively large pieces (50–200 kb). Endonucleases other than CAD have been postulated to perform this cleavage (Samejima et al., 2001; Susin et al., 2000). However, at least in our hands, no DNA degradation (whether to high-molecular-weight fragments or nucleosomal units) can be observed in cells lacking CAD (Kawane et al., 2003), indicating that other nucleases are not involved or play only a limited role.

Caspase cleavage also explains another hallmark of apoptotis, membrane blebbing. ROCK1 (Rho-associated kinase 1), a substrate of caspase 3, phosphorylates various cytoskeletal proteins, including myosin light chain, and regulates the actin cytoskeleton. ROCK1 is normally regulated by Rho GTPase, but its cleavage by caspase 3 removes its regulatory domain and renders it constitutively active (Coleman et al., 2001; Sebbagh et al., 2001). This aberrantly activated ROCK1 intensively phosphorylates myosin light chain, leading to membrane blebbing.

The microinjection of active CAD into cells causes DNA fragmentation and quickly kills the cells (Susin et al., 2000). In contrast, CAD-deficient cells are efficiently killed by apoptotic stimuli without DNA degradation (Kawane et al., 2003). As described above, given that many proteins and enzymes essential for cell survival are cleaved and inactivated by caspases, it is likely that once caspases are activated by apoptotic stimuli, there are many ways to kill the cells.

Engulfment of Apoptotic Cells

When apoptotic cells are left on a Petri dish for a long time, their plasma membrane ruptures and cellular contents are released, in a process called secondary necrosis (Kerr et al., 1972). On the other hand, apoptotic cells in vivo are quickly recognized by phagocytes and engulfed to prevent the release of their intracellular materials, which can be immunogenic. For the specific and efficient engulfment of apoptotic cells, the dead cells discharge molecules to

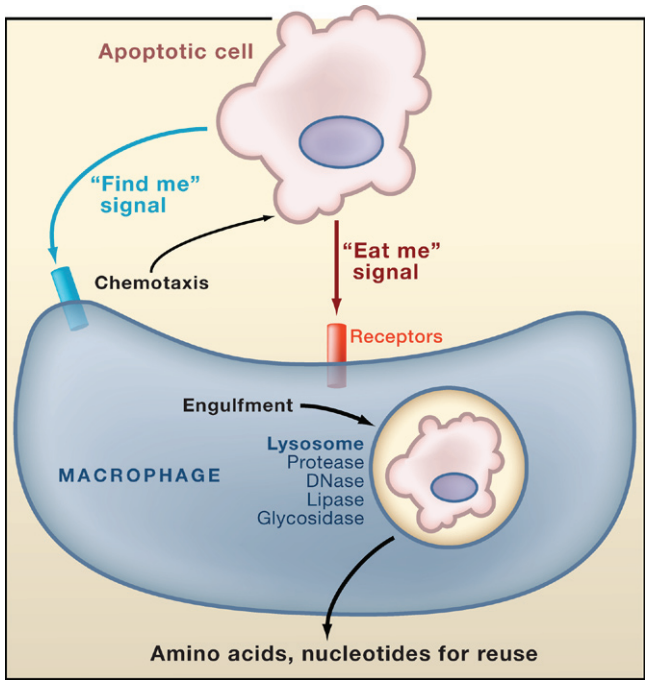

Figure 1. Engulfment of Apoptotic Cells by Macrophages
When cells undergo apoptosis, they release "find me" signals to recruit macrophages, and expose "eat me" signals on their surface. In response to the "find me" signal, macrophages approach the dead cells, and they engulf them by recognizing the "eat me" signal. The engulfed dead cells are transferred to lysosomes, where all their components are degraded into amino acids, nucleotides, fatty acids, and monosaccharides by lysosomal enzymes.

recruit phagocytes ("find me" signals), and they expose on their surface molecules that are recognized by phagocytes ("eat me" signals) (Figure 1).

"Find Me" Signals

By assaying the ability of the culture supernatant from apoptotic cells to trigger the chemotaxis of macrophages, Lauber et al. (2003) identified lysophosphatidylcholine (LPC) as a "find me" signal (Figure 2). It is released from apoptotic cells by the caspase-3-dependent activation of phospholipase A2, which converts phosphatidylcholine to LPC. The binding of LPC to G2A (G2 accumulation protein or G protein-coupled receptor 132) activates macrophages to undergo chemotaxis (Peter et al., 2008). This model is attractive, but the concentration of LPC required to cause the chemotaxis of phagocytes is rather high (20–30 μM) and may not be reached physiologically.

Two other molecules, sphingosine-1-phosphate (S1P) and CX3CL1/fractalkine, have also been proposed to act as "find me" signals (Gude et al., 2008; Truman et al., 2008). S1P is produced by sphingosine kinase in a caspase-dependent manner and secreted from apoptotic cells; it stimulates the chemotaxis of macrophages by binding its specific receptor, S1P-R. Fractalkine, CX3CL1, is synthesized as a membrane-associated protein, rapidly processed, and released from apoptotic neurons or B cells. It activates microglia and macrophages to undergo chemotaxis by binding to its receptor, CX3CR. In addition, ATP and UTP released from apoptotic cells in a caspase-dependent manner have recently been shown to act as "find me" signals for apoptotic cells (Elliott et al., 2009). Whether these proposed "find me" signals are redundant, additive, or synergistic remains to be studied.

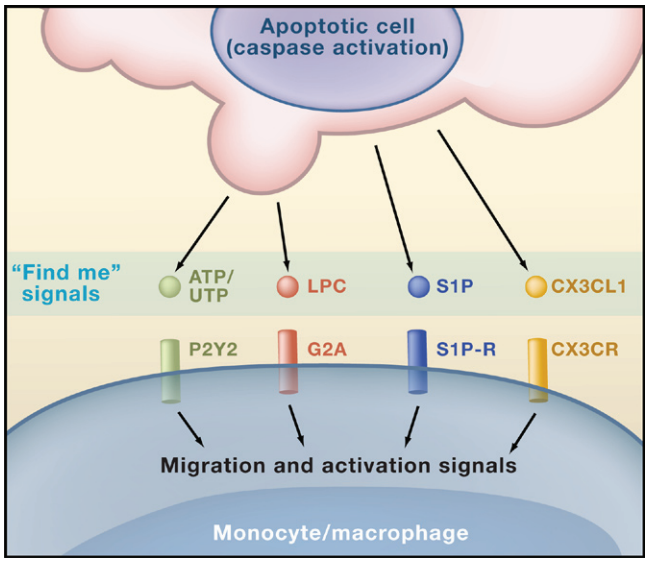

Figure 2. Proposed "Find Me" Signals
As "find me" signals, ATP/UTP, lysophosphatidylcholine (LPC), sphingosine-1-phosphate (S1P), and fractalkine CX3CL1 have been proposed. These molecules bind specific receptors on macrophages, all of which are G protein-coupled seven-transmembrane receptors, and activate them for chemotaxis.

Apoptotic cells appear to mostly (or exclusively) recruit macrophages (Truman et al., 2004). Yet, the proposed molecules (LPC, S1P, and ATP/UTP) activate not only macrophages but also neutrophils and lymphocytes (Florey and Haskard, 2009; Lecut et al., 2009). Bournazou et al. (2009) propose that lactoferrin is synthesized in apoptotic cells, secreted, and inhibits the migration of neutrophils. However, this may not be consistent with the quick killing of the death-factor-induced apoptosis that does not require protein synthesis. An involvement of lactoferrin in the "find me" process should be clarified with the lactoferrin-deficient mice (Ward et al., 2003).

"Eat Me" Signals

Macrophages engulf dead cells but not healthy ones, indicating either that dying cells expose "eat me" signals recognized by phagocytes or that healthy cells display "don't eat me" signals. The best-studied "eat me" signal is phosphatidylserine, a component of the cell plasma membrane that is kept exclusively on the inner leaflet of the lipid bilayer in healthy cells (Balasubramanian and Schroit, 2003). Phosphatidylserine is exposed on the cell surface when cells undergo apoptosis (Fadok et al., 1992). Moreover, when phosphatidylserine is inserted into the plasma membrane of erythrocytes, they are recognized and engulfed by macrophages (Tanaka and Schroit, 1983). Furthermore, the masking of phosphatidylserine inhibits the engulfment of apoptotic cells in vitro and in vivo (Asano et al., 2004; Krahling et al., 1999). These results strongly point to phosphatidylserine as the most likely candidate for the "eat me" signal.

The exposure of phosphatidylserine on the surface of apoptotic cells is found not only in mammals but also in *Drosophila* and *C. elegans* (van den Eijnde et al., 1998; Venegas and Zhou,

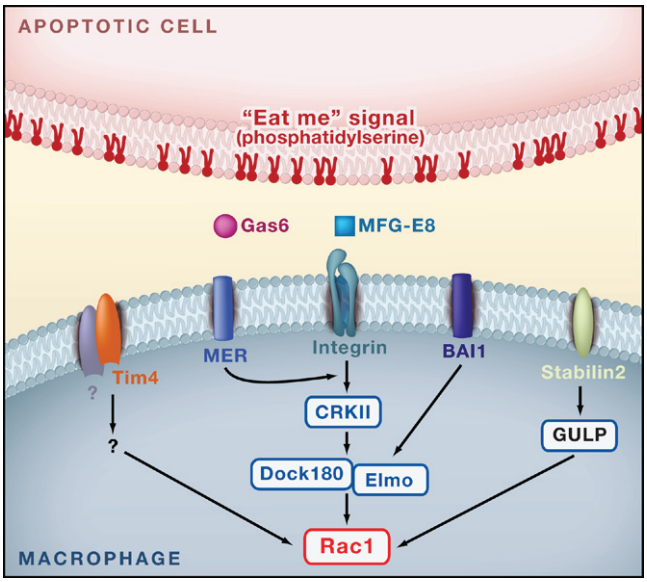

Figure 3. Molecules Proposed to Recognize Phosphatidylserine
The most likely "eat me" signal is phosphatidylserine. MFG-E8 and Gas6 are secreted proteins that bind phosphatidylserine and work as bridging molecules between apoptotic cells and macrophages. Tim-4, BAI1, and Stabilin-2 are type I-membrane proteins that are proposed phosphatidylserine receptors. Molecules that activate Rac1 (CrkII, Dock180, Elmo, and GULP) are involved in the engulfment of apoptotic cells.

2007). This process is caspase dependent (Martin et al., 1996), but how caspase activity leads to the cell-surface exposure of phosphatidylserine remains unsettled (Schlegel and Williamson, 2007). In one model, ATP-dependent translocases that maintain phosphatidylserine at the inner leaflet of the plasma membrane are inactivated in apoptotic cells, but Ca^{2+}-dependent phospholipid scamblase is activated, causing randomization of the membrane leaflet components (Balasubramanian and Schroit, 2003; Sahu et al., 2007). This model has been examined in mammals and *C. elegans*, but with controversial results (Darland-Ransom et al., 2008; Züllig et al., 2007).

CD47, also called integrin-associated protein (IAP), is a membrane protein with five membrane-spanning regions. When CD47-deficient red blood cells are injected into mice, they are more rapidly cleared by macrophages in the spleen than are CD47-positive cells. Oldenborg et al. (2000) therefore proposed that CD47 serves as a "don't eat me" signal. However, when CD47-positive red blood cells are loaded with phosphatidylserine, they are efficiently engulfed by macrophages (Tanaka and Schroit, 1983). Thymocytes express abundant CD47. When they undergo apoptosis, their CD47 expression is not lost, yet the apoptotic thymocytes are still efficiently engulfed by macrophages (Tada et al., 2003). These observations indicate that the "eat me" signal can overcome the "don't eat me" signal.

Bridging Molecules that Recognize Phosphatidylserine

Several secreted proteins have been identified as molecules that recognize the phosphatidylserine on apoptotic cells and promote their engulfment (Figure 3). Milk fat globule EGF factor 8 (MFG-E8), originally found associated with milk fat globules in mammary glands, is a secreted protein present on a subset of phagocytes that actively engulf apoptotic cells (Hanayama et al., 2002). It is expressed by macrophages and immature dendritic cells, including tingible-body macrophages and follicular dendritic cells at the germinal centers in the spleen and lymph nodes, thioglycollate-elicited peritoneal macrophages, granulocyte-macrophage colony stimulating factor (GM-CSF)-induced bone marrow-derived immature dendritic cells, and Langerhans cells in the skin (Hanayama et al., 2004; Kranich et al., 2008; Miyasaka et al., 2004). MFG-E8 contains one (human) or two (mouse) epidermal growth factor (EGF) domains in its N-terminal half, with the human and second mouse EGF domain carrying an RGD (Arg-Gly-Asp) motif. It has two factor-VIII-homologous domains (C1 and C2) in its C-terminal region. MFG-E8 associates with $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrin on phagocytes via its RGD motif (Yamaguchi et al., 2008), binds tightly to phosphatidylserine through its C1 and C2 domains, and stimulates the engulfment of apoptotic cells (Hanayama et al., 2002).

Two related proteins, growth arrest-specific 6 (Gas6) and protein S, which are abundant in plasma, bind phosphatidylserine (Nakano et al., 1997). TAM family members (Tyro3, Axl, and Mer), which are tyrosine-kinase receptors, are the receptors for Gas6 and protein S. Gas6 and protein S are involved in the vitamin K-dependent clotting system, and a deficiency in Gas6 or its receptor causes platelet dysfunction (Angelillo-Scherrer et al., 2005; Angelillo-Scherrer et al., 2001). On the other hand, mice expressing a kinase-dead mutant of Mer (Mer^{KD}) develop SLE-like autoimmunity (Scott et al., 2001), and the Gas6-TAM system has been proposed to play a role in the engulfment of apoptotic cells, particularly in the testis and retina (Prasad et al., 2006; Xiong et al., 2008). A recent report indicates that TAM receptors negatively regulate the innate immune reaction, and a lack of TAM receptors causes dendritic cells to overproduce interleukin-6 (IL-6), interferon (IFN), and TNFα (Rothlin et al., 2007). Given that SLE-type autoimmunity is regulated by cytokines, this overproduction of cytokines by dendritic cells might be responsible for the SLE-like autoimmunity found in the MER^{KD} mice (Scott et al., 2001).

Phosphatidylserine Receptors

Whether macrophages directly recognize apoptotic cells has been difficult to elucidate. A protein initially identified by Fadok et al. (2000) as a phosphatidylserine receptor (and consequently named PSR) is now reported to have a different function. Fadok et al. identified PSR by screening a phage-display library with a monoclonal antibody that inhibits the engulfment of apoptotic cells. Several groups subsequently reported that the deficiency of PSR causes the impaired engulfment of apoptotic cells, resulting in embryonic lethality in the mouse (Kunisaki et al., 2004; Li et al., 2003) and delayed engulfment of apoptotic cells in *C. elegans* (Wang et al., 2003). In contrast, Böse et al. (2004) who independently established *PSR* knockout mice, reported that PSR is not the protein recognized by the monoclonal antibody used by Fadok et al., and that PSR null macrophages have no defect in the engulfment of apoptotic cells. Subsequent reports have indicated that PSR is a chromatin-remodeling factor called Jumonji domain-containing 6 protein (JMJD6), which is present in the nucleus (Chang et al., 2007). Hence, the increased number of unengulfed apop-

Table 1. Molecules Involved in the Engulfment of Apoptotic Cells

C. elegans	Mammalian	Properties
CED-1	MEGF10	Type I membrane protein with multiple epidermal growth factor (EGF)-like domains in the extracellular region
CED-2	CrkII	Cytoplasmic protein with a Src homology 2 (SH2) and an SH3 domain, that functions as an adaptor for signal transduction
CED-5	Dock180	Cytoplasmic protein containing an SH3 domain; it associates with CrkII and ELMO, and activates the Rho family GTPase Rac1 as a guanine exchange factor
CED-6	GULP	Cytoplasmic protein with a phosphotyrosine-binding domain (PTB) and four SH3-binding motifs; it binds to CED-1 and functions upstream of CED-10
CED-7	ABC transporter	Protein with two homologous repeats, each harboring six transmembrane segments and one ATP-binding site
CED-10	Rac1	Small GTP-binding protein of the Ras superfamily
CED-12	ELMO	Cytoplasmic protein; it associates with CrkII and Dock180, and activates Rac1

totic cells in the animals lacking PSR may be due to increased cell death caused by the lack of JMJD6's chromatin remodeling function.

In investigating the mechanism by which macrophages that do not express MFG-E8 engulf apoptotic cells, we reported that type I membrane proteins called T cell immunoglobulin- and mucin-domain-containing molecule 4 (Tim-4) and Tim-1 serve as phosphatidylserine receptors (Miyanishi et al., 2007) (Figure 3). Tim-1 and Tim-4 consist of a signal sequence, an immunoglobulin V (IgV) domain, a mucin-like domain, a transmembrane domain, and a cytoplasmic region. They specifically bind phosphatidylserine with high affinity via their IgV domain. When Tim-1 or Tim-4 is expressed in mouse fibroblasts (NIH 3T3), which do not normally express Tim family members, the transformants efficiently engulf apoptotic cells. The short cytoplasmic region of Tim-4 is dispensable for the engulfment (Park et al., 2009) (M. Murai, M. Miyanishi, and S.N., unpublished data), indicating that Tim-4 associates with endogenous molecules on the fibroblast membrane to activate the engulfment signal. Among other Tim family members, Tim-3 also binds phosphatidylserine and stimulates the engulfment of apoptotic cells, although with less efficiency than Tim-1 or Tim-4 (Nakayama et al., 2009).

Tim-4 is expressed by macrophages and dendritic cells in the spleen, lymph nodes, thymus, and tonsils (Shakhov et al., 2004), and Tim-3 is expressed in $CD8^+$ dendritic cells in the spleen (Nakayama et al., 2009). These macrophages and dendritic cells are responsible for the engulfment of apoptotic cells and for the presentation of dead cell-associated antigens (Miyake et al., 2007). Tim-1, also called kidney injury molecule 1 (Kim-1), is expressed in kidney epithelial cells after ischemic injury (Ichimura et al., 2008) and in Th2 cells (Umetsu et al., 2005). In the kidney, Tim-1 is likely to be responsible for engulfing the damaged apoptotic or necrotic cell debris generated during ischemic injury, but the role of Tim-1 in Th2 cells is not clear. The Tim family genes are clustered on human chromosome 5q33.2 and mouse chromosome 11B1.1, which is the susceptible gene locus for the development of atopy (allergic hypersensitivity) and asthma (Kuchroo et al., 2003). Whether the newly identified function of Tim-1, Tim-3, and Tim-4 in apoptotic cell engulfment or the originally proposed function of Tim-1 and Tim-4 in the costimulation of T cells (Kuchroo et al., 2003) is responsible for this phenotype remains to be studied.

Park et al. (2007) report that brain-specific angiogenesis inhibitor 1 (BAI1), a member of the secretin/vasoactive intestinal polypeptide (VIP) receptor family with 7-transmembrane regions, is another potential phosphatidylserine receptor for apoptotic cells. BAI1 binds via thrombospondin type 1 repeats (TSPs) to phosphatidylserine, as well as to cardiolipin and other phospholipids. Its cytoplasmic region can interact with the signal transducer ELMO (see below) (Park et al., 2007). However, BAI1's possible function as a phosphatidylserine receptor for apoptotic cells seems to conflict with its neuron-specific expression in the brain (Mori et al., 2002). Another candidate phosphatidylserine receptor is stabilin-2, also called HARE (hyaluronic acid receptor for endocytosis), a type I membrane protein that carries a large extracellular region with seven fasciclin domains and fifteen EGF-like domains (Park et al., 2008). It is expressed by the sinusoidal endothelial cells of the spleen, lymph nodes, and bone marrow (Nonaka et al., 2007) and functions as a receptor for hyaluronic acids and heparin to regulate blood viscosity (Harris et al., 2008). How stabilin-2 accomplishes two jobs, as a phosphatidylserine receptor for apoptotic cells and as a scavenger receptor for hyaluronic acids, would be an interesting topic for study.

Signaling Pathways for Engulfment

Genetic analyses in *C. elegans* identified seven genes that mediate the recognition and engulfment of apoptotic cells in two parallel and partially redundant signaling pathways (the CED-1/-6/-7 and CED-2/-5/-10/-12 pathways) (Table 1) (Reddien and Horvitz, 2004). CED-1 is a transmembrane receptor that has multiple EGF-like domains in its extracellular region; it has high homology with mammalian multiple EGF-like-domains 10 (MEGF10) (Hamon et al., 2006) and may recognize phosphatidylserine on apoptotic cells (Venegas and Zhou, 2007). CED-6 is an ortholog of mammalian GULP (PTB [phosphotyrosine-binding] domain-containing engulfment adaptor protein) and binds to the intracellular domain of CED-1 (Su et al., 2002). CED-7 is homologous to the ABC transporters that actively transport a variety of substances across the plasma membrane and was originally suggested to be responsible for exposing the "eat me" signal on apoptotic cells. However, the ABC transporter CED-7 interacts with MEGF10 (CED-1) (Hamon et al., 2006), indicating that it functions in the engulfment process in phagocytes.

CED-2, -5, -10, and -12 correspond to mammalian CrkII, Dock180, Rac1, and ELMO1, respectively. CED-2/CrkII associates with CED-5/Dock180, a guanine-nucleotide exchange

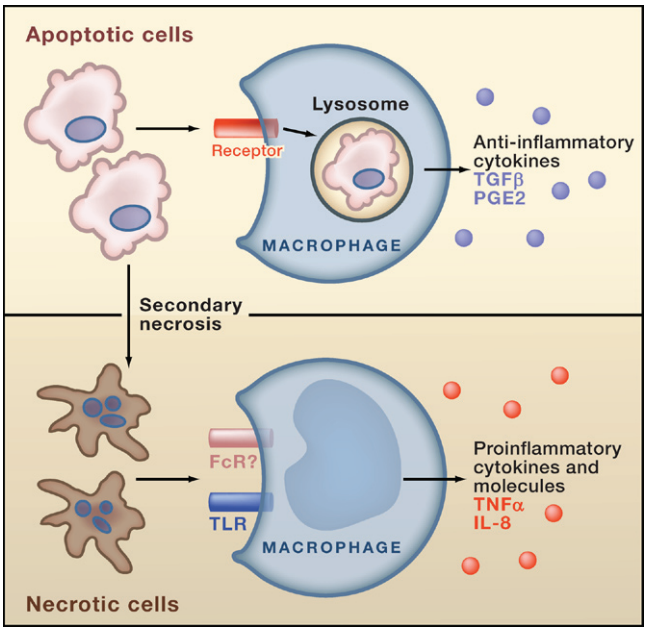

Figure 4. The Engulfment of Apoptotic versus Necrotic Cells
Macrophages engulfing apoptotic cells produce transforming growth factor β (TGFβ) and prostaglandin E2 (PGE2), which function as anti-inflammatory agents to inhibit the further recruitment of macrophages. When dead cells undergo secondary necrosis, the necrotic cells may activate macrophages through Fc receptor (FcR) and Toll-like receptors (TLRs) to produce inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin 8 (IL-8), which act to recruit more macrophages.

factor for CED-10/Rac1, and this interaction is positively regulated by CED-12/ELMO1 (Côté and Vuori, 2007). This pathway regulates actin polymerization and is involved not only in apoptotic-cell engulfment (Figure 3) but also in cell migration, neurite growth, and myoblast fusion. Integrin family members may act upstream of this pathway, but how apoptotic cells activate this pathway remains to be determined.

The engulfment of apoptotic cells is regulated by Rho family GTPases (Rac1, RhoA, Rab5, etc.) (Nakaya et al., 2006) and can be monitored at the molecular level by imaging using an actin-green fluorescent protein (GFP) fusion protein (Nakaya et al., 2008). This type of analysis indicates that the engulfment of apoptotic cells appears to occur at a limited number of portals in the phagocyte lamellipodia. A fluorescence resonance energy transfer (FRET) analysis for Rac1 indicates that the activation and deactivation of Rac1, controlled by "engulfment synapses," must be regulated with specific timing for the efficient engulfment of apoptotic cells. That is, when a phagocyte starts to engulf an apoptotic cell, activated Rac1 and integrin are recruited to the portal and induce the formation of phagocytic cups consisting of an actin patch. As soon as the dead cell sinks into the phagocyte through one of these cups, Rac1 is inactivated and the actin is depolymerized. Subsequently, Rab5 regulates the transfer of the dead-cell cargoes into lysosomes (Kitano et al., 2008).

The uptake of apoptotic cells by phagocytes induces the expression of transforming growth factor β (TGFβ) and IL-10 (Fadok et al., 2001) (Figure 4), which may inhibit the further recruitment of macrophages to the dying cells. On the other hand, if the dead cells persist in tissues, either because of impaired engulfment or because the number of apoptotic cells overwhelms the capacity of the phagocytes, the apoptotic cells undergo necrosis. When necrotic cells interact with or are engulfed by macrophages, the macrophages produce inflammatory cytokines (Fadok et al., 2001), which may recruit more macrophages as reinforcements. The activation of different cytokine genes upon their engulfment of apoptotic and necrotic cells suggests that the signal transduction pathways induced by these dead cells are different.

Autoimmune Disease Caused by the Inefficient Engulfment of Dead Cells

Systemic Lupus Erythematosus is a chronic autoimmune disease that causes a broad spectrum of clinical manifestations affecting the skin, kidney, lungs, blood vessels, and nervous system (D'Cruz et al., 2007). Patients with SLE have autoantibodies in their sera against nuclear components (anti-ribonucleoprotein and anti-DNA antibodies) and sometimes exhibit circulating DNA or nucleosomes (Rumore and Steinman, 1990). As unengulfed apoptotic cells are present in the germinal centers of the lymph nodes of some SLE patients and macrophages from these patients often show a reduced ability to engulf apoptotic cells, a deficiency in the clearance of apoptotic cells is proposed to be one of the causes of SLE (Gaipl et al., 2006).

MFG-E8-deficient female mice, particularly of the B6/129-mixed background, develop an age-dependent SLE type of autoimmune disease (Hanayama et al., 2004). These mice produce high concentrations of anti-double-stranded DNA and anti-nuclear antibodies and suffer from glomerular nephritis. When MFG-E8-deficient mice are immunized with keyhole limpet hemocyanin (KLH) to activate B lymphocytes, many apoptotic cells are left unengulfed on the tingible-body macrophages in the germinal centers, confirming that MFG-E8 has a nonredundant role in vivo in the engulfment of apoptotic cells by the tingible-body macrophages. It is likely that the unengulfed dead cells in MFG-E8-deficient mice undergo a secondary necrosis and release cellular components that activate the immune system to produce autoantibodies (Figure 5A). Like Fas-deficient lpr mice, in which autoreactive B cells are activated in a T cell-independent but Toll-like receptor (TLR)- and B cell receptor (BCR)-dependent mechanism (Herlands et al., 2008), the released cellular components may activate autoreactive B cells in a BCR- and TLR-dependent manner. This activation of autoreactive B cells may be further enhanced by cytokines produced by macrophages in response to stimulation by the necrotic cells. In any case, the MFG-E8-deficient mice provide a good model system for studying the molecular mechanisms by which endogenous cellular components activate the immune system extracellularly.

As described above, apoptotic cells are rapidly recognized and engulfed by macrophages at the early stage of their death process, mostly in a phosphatidylserine-dependent manner. On the other hand, how necrotic cells are recognized and engulfed by macrophages is not well elucidated. One likely system for clearing necrotic cells is the complement system (Trouw et al., 2008). C1q binds to dead cells at the later stages of apoptosis in an IgM-dependent manner (Ogden et al., 2005), and one of the signals on the dead cells for IgM-binding is lysophosphatidylcholine (Kim et al., 2002). Notably, in humans, almost all individuals deficient in

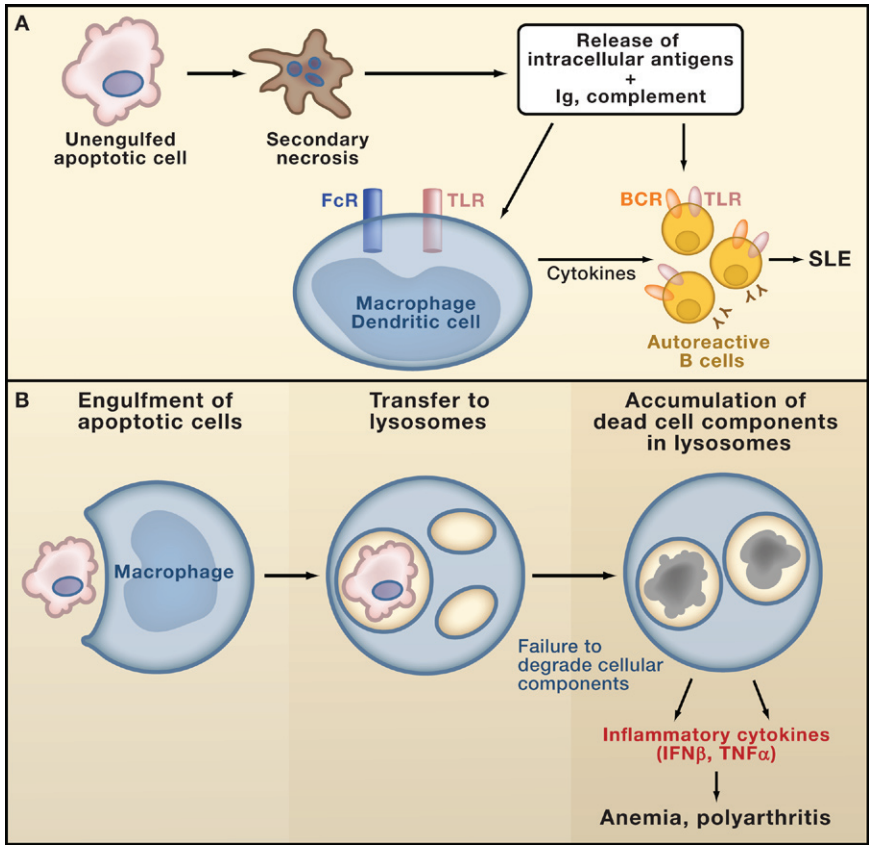

Figure 5. Immune System Activation by the Defective Engulfment of Apoptotic Cells

(A) Extracellular activation. If apoptotic cells are not swiftly engulfed, they undergo secondary necrosis, in which the plasma membrane is disintegrated, and the cellular components are released. Immunoglobulins and complement proteins bind to these cellular components and activate macrophages and B lymphocytes. In addition to FcR and B cell receptors (BCRs), Toll-like receptors (TLRs) appear to be involved in recognizing the cellular components and activating macrophages and B cells. The activated macrophages produce cytokines that will stimulate B cells to produce autoantibodies.

(B) Intracellular activation. After being engulfed by macrophages, dead cells are transferred to lysosomes and degraded. If the degradation does not occur properly, dead cell components accumulate in the lysosomes, leading to the intracellular activation of the innate immune system to produce proinflammatory cytokines such as interferon β (IFNβ) and tumor necrosis factor α (TNFα).

the *C1q* gene develop severe SLE (Botto and Walport, 2002). In C1q-deficient mice, unengulfed dead cells persist in the glomeruli of the kidneys, and the mice develop an SLE-like phenotype; this is particularly evident in the MRL/MP strain, which has a defect in the clearance of apoptotic cells (Potter et al., 2003). In this regard, the C1q-mediated engulfment may be a backup system for clearing dead cells, and it may be informative to cross C1q-deficient mice with MFG-E8-deficient mice.

Degradation of Apoptotic Cells in Macrophages

Activation of the Innate Immunity by Undigested DNA Left in Lysosomes

After apoptotic cells are engulfed by phagocytes, all of their components are degraded into amino acids, nucleotides, fatty acids, and monosaccharides in lysosomes. As described above, the chromosomal DNA of the apoptotic cells is degraded cell autonomously into 180 bp nucleosomal units by CAD and then further degraded in the lysosomes of macrophages. The enzyme that degrades the DNA of apoptotic cells in lysosomes is DNase II (Kawane et al., 2003), which functions under acidic conditions (Evans and Aguilera, 2003). DNase II is ubiquitously expressed in various tissues, particularly in macrophages. A lack of DNase II causes the accumulation of 180 bp fragmented DNA in macrophages (Kawane et al., 2001) and activates the macrophages to produce various cytokines. One of the cytokines produced by these macrophages is IFNβ, which is cytotoxic to erythroblasts and lymphocytes (Yoshida et al., 2005b) (Figure 5B).

Lethal Anemia and Polyarthritis Resulting from a Defect in DNA Degradation

Mice lacking DNase II die late in embryogenesis because of severe anemia (Kawane et al., 2001). Many TUNEL-positive erythroblasts can be found in the liver of mouse embryos lacking DNase II, and a deficiency in the *IFN-type I receptor* gene rescues their lethality (Yoshida et al., 2005b), indicating that the erythroblasts are killed by the action of IFNβ. Mice with a double deficiency for DNase II and IFN-type I receptor, or mice in which the *DNase II* gene is deleted after birth via an inducible conditional knockout strategy, develop polyarthritis as they age (Kawane et al., 2006). Their swollen joints show severe synovitis with aggressive pannus formation. The pannus carries osteoclasts at its leading edge, fills the joint cavity, erodes the cartilage, and destroys the bone. As in the joints of human rheumatoid arthritis patients, the genes for inflammatory cytokines (IL-1β, IL-6, and TNFα) are strongly activated in the affected joints. Human patients with rheumatoid arthritis are successfully treated with reagents that antagonize TNFα or IL-6 (Feldmann, 2002; Yokota et al., 2008). Similarly, the administration of an anti-TNFα antibody significantly improves the clinical score for the polyarthritis developed by the *DNase II* null mice (Kawane et al., 2006).

What triggers the rheumatoid arthritis in humans is unknown. In the *DNase II* null mice, macrophages carrying undigested DNA express *TNFα* mRNA (Kawane et al., 2006), and a low, but significant, level of TNFα is found in the serum before the joints show any abnormality. Given that *TNF*-transgenic mice, which constitutively produce a low level of TNFα, develop polyarthritis (Keffer et al., 1991), it is likely that the TNFα produced by the macrophages carrying undigested DNA is responsible for the development of the polyarthritis. Synovial cells respond to TNFα with high sensitivity to produce IL-1β and IL-6, which in turn stimulate the expression of the TNFα gene (Taberner et

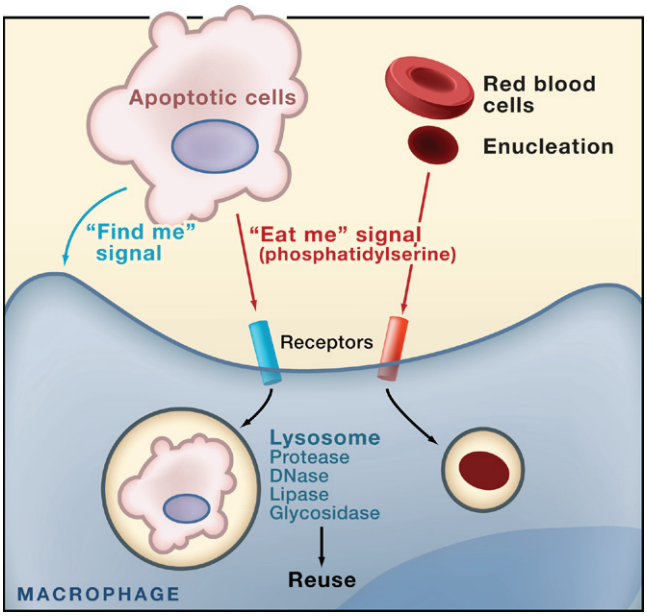

Figure 6. Engulfment of the Nuclei Expelled from Erythroid Precursor Cells

At the final stage of definitive erythropoiesis, an erythroblast undergoes unequal division into a reticulocyte and a nucleus surrounded by plasma membrane. Like apoptotic cells, the plasma membrane surrounding the nucleus exposes phosphatidylserine as an "eat me" signal and is engulfed by macrophages.

al., 2005; Zhang et al., 2004), causing a "cytokine storm" in the joint. This leads to the growth of synovial cells, pannus formation, and the development of polyarthritis (Migita et al., 2001).

The pathologies (anemia and polyarthritis) caused by a deficiency of DNase II are examples of lysosomal storage diseases, which are diseases caused by the inactivation or malfunction of lysosomal enzymes, including proteases, glycosidases, and lipases (Neufeld, 1991). Proteins, polysaccharides, DNA, and RNA of bacterial or viral origin activate the innate immunity to produce various cytokines (Uematsu and Akira, 2007). The results from the *DNase II* null mice indicate that mammalian DNA that accumulates in the lysosomes of macrophages also activates the innate immune response. Other cellular components that escape degradation in the lysosomes may also activate the *TNF*α and *IFN*β genes. The fact that cytokines are constitutively secreted by macrophages lacking lysosomal acid lipase (Lian et al., 2004), and by fibroblasts derived from patients with Niemann-Pick Disease Type C (Suzuki et al., 2007), an inherited lipid storage disorder, may support this notion. Some patients with rheumatoid arthritis can be cured by bone marrow transplantation (Ikehara, 2002), suggesting that these patients have a defect(s) in bone-marrow-derived cells. Determining whether these patients have lysosomal enzyme defects will be useful for improving their treatment.

Signaling from DNA to Cytokine Gene Expression

Cells that are infected by viruses or bacteria normally produce IFNβ and TNFα (Honda et al., 2006). There are two pathways by which pathogens activate the cytokine genes. In one, TLR recognizes pathogens extracellularly and transduces signals via the adaptor proteins MyD88 and TRIF to activate the transcription factors IFN-regulatory factor (IRF)3/IRF7 and NF-κB, which induce IFNβ and TNFα. In the other pathway, RIG-I/MDA5 recognizes intracellular pathogens and activates IRF3/IRF7 and NF-κB via an adaptor called IPS-1. The expression of the IFNβ and TNFα genes in the macrophages lacking DNase II is not blocked by a deficiency in the TLR system, indicating that the mammalian DNA that accumulates in the lysosomes activates the innate immune system in a TLR-independent manner (Okabe et al., 2005). We recently found that mammalian DNA in lysosomes activates *TNF*α and *IFN*β gene expression through the system for intracellular pathogens and that this system can be regulated by Janus phosphatases called Eyes absent (Eya) (Okabe et al., 2009). Eya binds to IPS-1 and is involved not only in the mammalian DNA-mediated innate immune reaction but also in the virus-induced one, indicating that endogenous DNA and viruses intracellularly activate the innate immune response using a similar mechanism. Identification of the targets of Eya's phosphatase will contribute to the understanding how the intracellular pathogens activate innate immunity.

Engulfment and Degradation of Nuclei from Erythroid Precursors

Early in mammalian embryogenesis, red blood cells are produced in the yolk sac in a process called primitive erythropoiesis. Erythropoiesis then takes place in the fetal liver at later stages of embryogenesis and in the bone marrow after birth, and this process is called definitive erythropoiesis. Unlike the nucleated erythroid cells produced in the yolk sac, those produced in the fetal liver and bone marrow are enucleated. The definitive erythropoiesis in both the bone marrow and fetal liver takes place in anatomical units called erythroblastic islands. At the center of each island, there is a macrophage that supports the proliferation and differentiation of the erythroid precursor cells (Chasis and Mohandas, 2008). At the final stage of erythropoiesis, the erythroid cells autonomously undergo enucleation, and the expelled nuclei are engulfed by the central macrophage, suggesting that the expelled nuclei also expose an "eat me" signal on their surface (Figure 6).

The engulfment of expelled nuclei by macrophages has been shown to be phosphatidylserine dependent in experiments using nuclei collected from cultured erythroid precursor cells that spontaneously undergo enucleation (Yoshida et al., 2005a). Immediately after a nucleus is separated from its reticulocyte, phosphatidylserine is exposed on the outer leaflet of the plasma membrane surrounding the nucleus. It is likely that the plasma membrane cannot maintain its integrity because of a lack of ATP, because once separated from the reticulocyte, the nucleus loses its sources of new ATP (mitochondria and glycolysis). It is not yet known what molecules in the macrophages of the fetal liver and bone marrow are involved in recognizing the phosphatidylserine on the nuclei and engulfing them.

Every day, 2×10^{11} new red blood cells are produced in a human adult, meaning that this number of nuclei needs to be phagocytosed. This is at least ten times the number of dead cells. If nuclei, which are highly immunogenic, are released into the circulation because of inefficient engulfment, they will activate the immune system. In DNase II-deficient mice,

the macrophages at the erythroblastic islands in the fetal liver and bone marrow carry a number of undigested nuclei in their lysosomes, indicating that DNase II is responsible for degrading the DNA from the engulfed erythroblast nuclei (Kawane et al., 2001). This means that, each day, the DNA from 2×10^{11} erythroblasts in a human adult, corresponding to about 1.0 g, is degraded by a single enzyme, DNase II, in macrophages. As described above for the DNA of dead cells, the inefficient digestion of the nuclear DNA from erythroid precursors can also cause severe inflammation.

Future Prospects

To combat bacterial and viral infection, mammals have developed a sophisticated immune system, which includes the acquired and innate immune systems. In the innate immune system, macrophages and dendritic cells recognize pathogens extracellularly and intracellularly and produce various cytokines such as IFNβ, IL-1β, and TNFα to contend with the pathogens directly. These cytokines also activate the acquired immune response to produce antibodies and cytotoxicity to combat the pathogens. Endogenous components derived from dead cells can also activate both the acquired and innate immune systems. Undigested DNA that accumulates in the lysosomes of macrophages can activate the intracellular signaling pathway for the innate immune reaction, while cellular components released from dead cells appear to activate the immune reaction extracellularly, through the BCR and TLR system. The immune reaction triggered by bacterial and viral pathogens is transient; that is, when the bacteria or viruses are removed by the action of IFN or TNF, the immune reaction ceases. In contrast, if a defect in the engulfment or digestion of dead cells or erythroid nuclei is not repaired, the immune system will be chronically activated, which may be responsible for SLE-type autoimmune disease and polyarthritis, two major autoimmune diseases in humans. Our knowledge about how dead cells and the expelled erythroid nuclei are recognized, engulfed, transferred to lysosomes, and degraded is still very primitive. The elucidation of these processes will help us understand the pathophysiology of various human diseases, especially autoimmune diseases, and will lead to the development of new therapeutic strategies for treating them.

Apoptotic-cell engulfment is phosphatidylserine dependent. Other examples of phosphatidylserine-dependent processes are the involution of mammary glands (Hanayama and Nagata, 2005) and the axon pruning that takes place during the development of neural circuits (Awasaki et al., 2006). In the involution of mammary glands, milk fat globules remaining in the mammary glands are re-absorbed by mammary epithelial cells in a phosphatidylserine-dependent manner (Hanayama and Nagata, 2005). MFG-E8, which is secreted from mammary epithelial cells, promotes the reabsorption of milk fat globules. The failure of this process induces mastitis, another case in which the inefficient clearance of unnecessary cellular components can lead to inflammation. In axon pruning, unnecessary or extra axons degenerate, expose phosphatidylserine, and are engulfed by glia. However, how the glial phagocytes recognize the phosphatidylserine on the pruned axons remains to be elucidated.

Finally, the main reason for the engulfment of dead cells is to degrade their intracellular materials before their cellular contents are released, which could activate the immune system. However, in some cases, phagocytes actively induce programmed cell death. For example, macrophages within the developing eye induce the programmed cell death of the vascular endothelial cells via Wnt ligand (Lobov et al., 2005), and phagocyte-induced programmed cell death serves as a backup death-inducing system in *C. elegans* (Reddien and Horvitz, 2004). How macrophages are induced to engulf apparently healthy cells is a mystery. The elucidation of this mechanism may help explain hemophagocytic syndrome, in which activated macrophages engulf apparently normal red blood cells and other cells.

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REFERENCES

Angelillo-Scherrer, A., de Frutos, P., Aparicio, C., Melis, E., Savi, P., Lupu, F., Arnout, J., Dewerchin, M., Hoylaerts, M., Herbert, J., et al. (2001). Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. Nat. Med. *7*, 215–221.

Angelillo-Scherrer, A., Burnier, L., Flores, N., Savi, P., DeMol, M., Schaeffer, P., Herbert, J.M., Lemke, G., Goff, S.P., Matsushima, G.K., et al. (2005). Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. J. Clin. Invest. *115*, 237–246.

Asano, K., Miwa, M., Miwa, K., Hanayama, R., Nagase, H., Nagata, S., and Tanaka, M. (2004). Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. J. Exp. Med. *200*, 459–467.

Awasaki, T., Tatsumi, R., Takahashi, K., Arai, K., Nakanishi, Y., Ueda, R., and Ito, K. (2006). Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during Drosophila metamorphosis. Neuron *50*, 855–867.

Balasubramanian, K., and Schroit, A.J. (2003). Aminophospholipid asymmetry: a matter of life and death. Annu. Rev. Physiol. *65*, 701–734.

Bassnett, S. (2002). Lens organelle degradation. Exp. Eye Res. *74*, 1–6.

Böse, J., Gruber, A.D., Helming, L., Schiebe, S., Wegener, I., Hafner, M., Beales, M., Köntgen, F., and Lengeling, A. (2004). The phosphatidylserine receptor has essential functions during embryogenesis but not in apoptotic cell removal. J. Biol. *3*, 15.

Botto, M., and Walport, M.J. (2002). C1q, autoimmunity and apoptosis. Immunobiology *205*, 395–406.

Bournazou, I., Pound, J.D., Duffin, R., Bournazos, S., Melville, L.A., Brown, S.B., Rossi, A.G., and Gregory, C.D. (2009). Apoptotic human cells inhibit migration of granulocytes via release of lactoferrin. J. Clin. Invest. *119*, 20–32.

Chang, B., Chen, Y., Zhao, Y., and Bruick, R.K. (2007). JMJD6 is a histone arginine demethylase. Science *318*, 444–447.

Chasis, J.A., and Mohandas, N. (2008). Erythroblastic islands: niches for erythropoiesis. Blood *112*, 470–478.

Cho, Y.S., Challa, S., Moquin, D., Genga, R., Ray, T.D., Guildford, M., and Chan, F.K. (2009). Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell

137, 1112–1123.

Coleman, M.L., Sahai, E.A., Yeo, M., Bosch, M., Dewar, A., and Olson, M.F. (2001). Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. Nat. Cell Biol. *3*, 339–345.

Côté, J.F., and Vuori, K. (2007). GEF what? Dock180 and related proteins help Rac to polarize cells in new ways. Trends Cell Biol. *17*, 383–393.

D'Cruz, D.P., Khamashta, M.A., and Hughes, G.R. (2007). Systemic lupus erythematosus. Lancet *369*, 587–596.

Darland-Ransom, M., Wang, X., Sun, C.L., Mapes, J., Gengyo-Ando, K., Mitani, S., and Xue, D. (2008). Role of C. elegans TAT-1 protein in maintaining plasma membrane phosphatidylserine asymmetry. Science *320*, 528–531.

Elliott, M.R., Chekeni, F.B., Trampont, P.C., Lazarowski, E.R., Kadl, A., Walk, S.F., Park, D., Woodson, R.I., Ostankovich, M., Sharma, P., et al. (2009). Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature *461*, 282–286.

Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., and Nagata, S. (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature *391*, 43–50.

Evans, C.J., and Aguilera, R.J. (2003). DNase II: genes, enzymes and function. Gene *322*, 1–15.

Fadok, V.A., Voelker, D.R., Campbell, P.A., Cohen, J.J., Bratton, D.L., and Henson, P.M. (1992). Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J. Immunol. *148*, 2207–2216.

Fadok, V.A., Bratton, D.L., Rose, D.M., Pearson, A., Ezekewitz, R.A., and Henson, P.M. (2000). A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature *405*, 85–90.

Fadok, V.A., Bratton, D.L., Guthrie, L., and Henson, P.M. (2001). Differential effects of apoptotic versus lysed cells on macrophage production of cytokines: role of proteases. J. Immunol. *166*, 6847–6854.

Feldmann, M. (2002). Development of anti-TNF therapy for rheumatoid arthritis. Nat. Rev. Immunol. *2*, 364–371.

Florey, O., and Haskard, D.O. (2009). Sphingosine 1-phosphate enhances Fc gamma receptor-mediated neutrophil activation and recruitment under flow conditions. J. Immunol. *183*, 2330–2336.

Gaipl, U.S., Kuhn, A., Sheriff, A., Munoz, L.E., Franz, S., Voll, R.E., Kalden, J.R., and Herrmann, M. (2006). Clearance of apoptotic cells in human SLE. Curr. Dir. Autoimmun. *9*, 173–187.

Gude, D.R., Alvarez, S.E., Paugh, S.W., Mitra, P., Yu, J., Griffiths, R., Barbour, S.E., Milstien, S., and Spiegel, S. (2008). Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a "come-and-get-me" signal. FASEB J. *22*, 2629–2638.

Hamon, Y., Trompier, D., Ma, Z., Venegas, V., Pophillat, M., Mignotte, V., Zhou, Z., and Chimini, G. (2006). Cooperation between engulfment receptors: the case of ABCA1 and MEGF10. PLoS ONE *1*, e120.

Hanayama, R., and Nagata, S. (2005). Impaired involution of mammary glands in the absence of milk fat globule EGF factor 8. Proc. Natl. Acad. Sci. USA *102*, 16886–16891.

Hanayama, R., Tanaka, M., Miwa, K., Shinohara, A., Iwamatsu, A., and Nagata, S. (2002). Identification of a factor that links apoptotic cells to phagocytes. Nature *417*, 182–187.

Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y., and Nagata, S. (2004). Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science *304*, 1147–1150.

Harris, E.N., Weigel, J.A., and Weigel, P.H. (2008). The human hyaluronan receptor for endocytosis (HARE/Stabilin-2) is a systemic clearance receptor for heparin. J. Biol. Chem. *283*, 17341–17350.

He, S., Wang, L., Miao, L., Wang, T., Du, F., Zhao, L., and Wang, X. (2009). Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell *137*, 1100–1111.

Herlands, R.A., Christensen, S.R., Sweet, R.A., Hershberg, U., and Shlomchik, M.J. (2008). T cell-independent and toll-like receptor-dependent antigen-driven activation of autoreactive B cells. Immunity *29*, 249–260.

Honda, K., Takaoka, A., and Taniguchi, T. (2006). Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. Immunity *25*, 349–360.

Ichimura, T., Asseldonk, E.J., Humphreys, B.D., Gunaratnam, L., Duffield, J.S., and Bonventre, J.V. (2008). Kidney injury molecule-1 is a phosphatidylserine receptor that confers a phagocytic phenotype on epithelial cells. J. Clin. Invest. *118*, 1657–1668.

Ikehara, S. (2002). Bone marrow transplantation: a new strategy for intractable diseases. Drugs Today (Barc) *38*, 103–111.

Kawane, K., Fukuyama, H., Kondoh, G., Takeda, J., Ohsawa, Y., Uchiyama, Y., and Nagata, S. (2001). Requirement of DNase II for definitive erythropoiesis in the mouse fetal liver. Science *292*, 1546–1549.

Kawane, K., Fukuyama, H., Yoshida, H., Nagase, H., Ohsawa, Y., Uchiyama, Y., Okada, K., Iida, T., and Nagata, S. (2003). Impaired thymic development in mouse embryos deficient in apoptotic DNA degradation. Nat. Immunol. *4*, 138–144.

Kawane, K., Ohtani, M., Miwa, K., Kizawa, T., Kanbara, Y., Yoshioka, Y., Yoshikawa, H., and Nagata, S. (2006). Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. Nature *443*, 998–1002.

Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., and Kollias, G. (1991). Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. *10*, 4025–4031.

Kerr, J.F., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer *26*, 239–257.

Kim, S.J., Gershov, D., Ma, X., Brot, N., and Elkon, K.B. (2002). I-PLA(2) activation during apoptosis promotes the exposure of membrane lysophosphatidylcholine leading to binding by natural immunoglobulin M antibodies and complement activation. J. Exp. Med. *196*, 655–665.

Kitano, M., Nakaya, M., Nakamura, T., Nagata, S., and Matsuda, M. (2008). Imaging of Rab5 activity identifies essential regulators for phagosome maturation. Nature *453*, 241–245.

Krahling, S., Callahan, M.K., Williamson, P., and Schlegel, R.A. (1999). Exposure of phosphatidylserine is a general feature in the phagocytosis of apoptotic lymphocytes by macrophages. Cell Death Differ. *6*, 183–189.

Krammer, P.H. (2000). CD95's deadly mission in the immune system. Nature *407*, 789–795.

Kranich, J., Krautler, N.J., Heinen, E., Polymenidou, M., Bridel, C., Schildknecht, A., Huber, C., Kosco-Vilbois, M.H., Zinkernagel, R., Miele, G., and Aguzzi, A. (2008). Follicular dendritic cells control engulfment of apoptotic bodies by secreting Mfge8. J. Exp. Med. *205*, 1293–1302.

Kroemer, G., and Levine, B. (2008). Autophagic cell death: the story of a misnomer. Nat. Rev. Mol. Cell Biol. *9*, 1004–1010.

Kroemer, G., Galluzzi, L., Vandenabeele, P., Abrams, J., Alnemri, E.S., Baehrecke, E.H., Blagosklonny, M.V., El-Deiry, W.S., Golstein, P., Green, D.R., et al; Nomenclature Committee on Cell Death 2009. (2009). Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ. *16*, 3–11.

Kuchroo, V.K., Umetsu, D.T., DeKruyff, R.H., and Freeman, G.J. (2003). The TIM gene family: emerging roles in immunity and disease. Nat. Rev. Immunol. *3*, 454–462.

Kunisaki, Y., Masuko, S., Noda, M., Inayoshi, A., Sanui, T., Harada, M., Sasazuki, T., and Fukui, Y. (2004). Defective fetal liver erythropoiesis and T lymphopoiesis in mice lacking the phosphatidylserine receptor. Blood *103*, 3362–3364.

Lauber, K., Bohn, E., Kröber, S.M., Xiao, Y.J., Blumenthal, S.G., Lindemann, R.K., Marini, P., Wiedig, C., Zobywalski, A., Baksh, S., et al. (2003). Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell *113*, 717–730.

Lecut, C., Frederix, K., Johnson, D.M., Deroanne, C., Thiry, M., Faccinetto, C., Marée, R., Evans, R.J., Volders, P.G., Bours, V., and Oury, C. (2009). P2X1 ion channels promote neutrophil chemotaxis through Rho kinase activation. J. Immunol. *183*, 2801–2809.

Li, M.O., Sarkisian, M.R., Mehal, W.Z., Rakic, P., and Flavell, R.A. (2003). Phosphatidylserine receptor is required for clearance of apoptotic cells. Science *302*, 1560–1563.

Lian, X., Yan, C., Yang, L., Xu, Y., and Du, H. (2004). Lysosomal acid lipase deficiency causes respiratory inflammation and destruction in the lung. Am. J. Physiol. Lung Cell. Mol. Physiol. *286*, L801–L807.

Lippens, S., Denecker, G., Ovaere, P., Vandenabeele, P., and Declercq, W. (2005). Death penalty for keratinocytes: apoptosis versus cornification. Cell Death Differ. *12* (*Suppl 2*), 1497–1508.

Liu, X., Zou, H., Slaughter, C., and Wang, X. (1997). DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. Cell *89*, 175–184.

Lobov, I.B., Rao, S., Carroll, T.J., Vallance, J.E., Ito, M., Ondr, J.K., Kurup, S., Glass, D.A., Patel, M.S., Shu, W., et al. (2005). WNT7b mediates macrophage-induced programmed cell death in patterning of the vasculature. Nature *437*, 417–421.

Lockshin, R.A., and Zakeri, Z. (2001). Programmed cell death and apoptosis: origins of the theory. Nat. Rev. Mol. Cell Biol. *2*, 545–550.

Lüthi, A.U., and Martin, S.J. (2007). The CASBAH: a searchable database of caspase substrates. Cell Death Differ. *14*, 641–650.

Martin, S.J., Finucane, D.M., Amarante-Mendes, G.P., O'Brien, G.A., and Green, D.R. (1996). Phosphatidylserine externalization during CD95-induced apoptosis of cells and cytoplasts requires ICE/CED-3 protease activity. J. Biol. Chem. *271*, 28753–28756.

Migita, K., Tanaka, F., Yamasaki, S., Shibatomi, K., Ida, H., Kawakami, A., Aoyagi, T., Kawabe, Y., and Eguchi, K. (2001). Regulation of rheumatoid synoviocyte proliferation by endogenous p53 induction. Clin. Exp. Immunol. *126*, 334–338.

Miyake, Y., Asano, K., Kaise, H., Uemura, M., Nakayama, M., and Tanaka, M. (2007). Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J. Clin. Invest. *117*, 2268–2278.

Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T., and Nagata, S. (2007). Identification of Tim4 as a phosphatidylserine receptor. Nature *450*, 435–439.

Miyasaka, K., Hanayama, R., Tanaka, M., and Nagata, S. (2004). Expression of milk fat globule epidermal growth factor 8 in immature dendritic cells for engulfment of apoptotic cells. Eur. J. Immunol. *34*, 1414–1422.

Mori, K., Kanemura, Y., Fujikawa, H., Nakano, A., Ikemoto, H., Ozaki, I., Matsumoto, T., Tamura, K., Yokota, M., and Arita, N. (2002). Brain-specific angiogenesis inhibitor 1 (BAI1) is expressed in human cerebral neuronal cells. Neurosci. Res. *43*, 69–74.

Nagata, S. (1997). Apoptosis by death factor. Cell *88*, 355–365.

Nakano, T., Ishimoto, Y., Kishino, J., Umeda, M., Inoue, K., Nagata, K., Ohashi, K., Mizuno, K., and Arita, H. (1997). Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. J. Biol. Chem. *272*, 29411–29414.

Nakaya, M., Tanaka, M., Okabe, Y., Hanayama, R., and Nagata, S. (2006). Opposite effects of rho family GTPases on engulfment of apoptotic cells by macrophages. J. Biol. Chem. *281*, 8836–8842.

Nakaya, M., Kitano, M., Matsuda, M., and Nagata, S. (2008). Spatiotemporal activation of Rac1 for engulfment of apoptotic cells. Proc. Natl. Acad. Sci. USA *105*, 9198–9203.

Nakayama, M., Akiba, H., Takeda, K., Kojima, Y., Hashiguchi, M., Azuma, M., Yagita, H., and Okumura, K. (2009). Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. Blood *113*, 3821–3830.

Neufeld, E.F. (1991). Lysosomal storage diseases. Annu. Rev. Biochem. *60*, 257–280.

Nonaka, H., Tanaka, M., Suzuki, K., and Miyajima, A. (2007). Development of murine hepatic sinusoidal endothelial cells characterized by the expression of hyaluronan receptors. Dev. Dyn. *236*, 2258–2267.

Oberst, A., Bender, C., and Green, D.R. (2008). Living with death: the evolution of the mitochondrial pathway of apoptosis in animals. Cell Death Differ. *15*, 1139–1146.

Ogden, C.A., Kowalewski, R., Peng, Y., Montenegro, V., and Elkon, K.B. (2005). IGM is required for efficient complement mediated phagocytosis of apoptotic cells in vivo. Autoimmunity *38*, 259–264.

Ohsumi, Y. (2001). Molecular dissection of autophagy: two ubiquitin-like systems. Nat. Rev. Mol. Cell Biol. *2*, 211–216.

Okabe, Y., Kawane, K., Akira, S., Taniguchi, T., and Nagata, S. (2005). Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation. J. Exp. Med. *202*, 1333–1339.

Okabe, Y., Sano, T., and Nagata, S. (2009). Regulation of the innate immune response by threonine-phosphatase of Eyes absent. Nature *460*, 520–524.

Oldenborg, P.A., Zheleznyak, A., Fang, Y.F., Lagenaur, C.F., Gresham, H.D., and Lindberg, F.P. (2000). Role of CD47 as a marker of self on red blood cells. Science *288*, 2051–2054.

Ow, Y.P., Green, D.R., Hao, Z., and Mak, T.W. (2008). Cytochrome c: functions beyond respiration. Nat. Rev. Mol. Cell Biol. *9*, 532–542.

Park, D., Tosello-Trampont, A.C., Elliott, M.R., Lu, M., Haney, L.B., Ma, Z., Klibanov, A.L., Mandell, J.W., and Ravichandran, K.S. (2007). BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature *450*, 430–434.

Park, S.Y., Jung, M.Y., Kim, H.J., Lee, S.J., Kim, S.Y., Lee, B.H., Kwon, T.H., Park, R.W., and Kim, I.S. (2008). Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell Death Differ. *15*, 192–201.

Park, D., Hochreiter-Hufford, A., and Ravichandran, K.S. (2009). The phosphatidylserine receptor TIM-4 does not mediate direct signaling. Curr. Biol. *19*, 346–351.

Peter, C., Waibel, M., Radu, C.G., Yang, L.V., Witte, O.N., Schulze-Osthoff, K., Wesselborg, S., and Lauber, K. (2008). Migration to apoptotic "find-me" signals is mediated via the phagocyte receptor G2A. J. Biol. Chem. *283*, 5296–5305.

Potter, P.K., Cortes-Hernandez, J., Quartier, P., Botto, M., and Walport, M.J. (2003). Lupus-prone mice have an abnormal response to thioglycolate and an impaired clearance of apoptotic cells. J. Immunol. *170*, 3223–3232.

Prasad, D., Rothlin, C.V., Burrola, P., Burstyn-Cohen, T., Lu, Q., Garcia de Frutos, P., and Lemke, G. (2006). TAM receptor function in the retinal pigment epithelium. Mol. Cell. Neurosci. *33*, 96–108.

Ravichandran, K.S., and Lorenz, U. (2007). Engulfment of apoptotic cells: signals for a good meal. Nat. Rev. Immunol. *7*, 964–974.

Reddien, P.W., and Horvitz, H.R. (2004). The engulfment process of programmed cell death in caenorhabditis elegans. Annu. Rev. Cell Dev. Biol. *20*, 193–221.

Rothlin, C.V., Ghosh, S., Zuniga, E.I., Oldstone, M.B., and Lemke, G. (2007). TAM receptors are pleiotropic inhibitors of the innate immune response. Cell *131*, 1124–1136.

Rumore, P.M., and Steinman, C.R. (1990). Endogenous circulating DNA in systemic lupus erythematosus. Occurrence as multimeric complexes bound to histone. J. Clin. Invest. *86*, 69–74.

Sahu, S.K., Gummadi, S.N., Manoj, N., and Aradhyam, G.K. (2007). Phospholipid scramblases: an overview. Arch. Biochem. Biophys. *462*, 103–114.

Sakahira, H., Enari, M., and Nagata, S. (1998). Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature *391*, 96–99.

Sakahira, H., Iwamatsu, A., and Nagata, S. (2000). Specific chaperone-like activity of inhibitor of caspase-activated DNase for caspase-activated DNase. J. Biol. Chem. *275*, 8091–8096.

Sakahira, H., Takemura, Y., and Nagata, S. (2001). Enzymatic active site of caspase-activated DNase (CAD) and its inhibition by inhibitor of CAD. Arch. Biochem. Biophys. *388*, 91–99.

Samejima, K., Tone, S., and Earnshaw, W.C. (2001). CAD/DFF40 nuclease is dispensable for high molecular weight DNA cleavage and stage I chromatin condensation in apoptosis. J. Biol. Chem. *276*, 45427–45432.

Schlegel, R.A., and Williamson, P. (2007). P.S. to PS (phosphatidylserine)—pertinent proteins in apoptotic cell clearance. Sci. STKE *2007*, pe57.

Scott, R.S., McMahon, E.J., Pop, S.M., Reap, E.A., Caricchio, R., Cohen, P.L., Earp, H.S., and Matsushima, G.K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. Nature *411*, 207–211.

Sebbagh, M., Renvoizé, C., Hamelin, J., Riché, N., Bertoglio, J., and Bréard, J. (2001). Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. Nat. Cell Biol. *3*, 346–352.

Shakhov, A.N., Rybtsov, S., Tumanov, A.V., Shulenin, S., Dean, M., Kuprash, D.V., and Nedospasov, S.A. (2004). SMUCKLER/TIM4 is a distinct member of TIM family expressed by stromal cells of secondary lymphoid tissues and associated with lymphotoxin signaling. Eur. J. Immunol. *34*, 494–503.

Strasser, A., Jost, P.J., and Nagata, S. (2009). The many roles of FAS receptor signaling in the immune system. Immunity *30*, 180–192.

Su, H.P., Nakada-Tsukui, K., Tosello-Trampont, A.C., Li, Y., Bu, G., Henson, P.M., and Ravichandran, K.S. (2002). Interaction of CED-6/GULP, an adapter protein involved in engulfment of apoptotic cells with CED-1 and CD91/low density lipoprotein receptor-related protein (LRP). J. Biol. Chem. *277*, 11772–11779.

Susin, S.A., Daugas, E., Ravagnan, L., Samejima, K., Zamzami, N., Loeffler, M., Costantini, P., Ferri, K.F., Irinopoulou, T., Prévost, M.C., et al. (2000). Two distinct pathways leading to nuclear apoptosis. J. Exp. Med. *192*, 571–580.

Suzuki, M., Sugimoto, Y., Ohsaki, Y., Ueno, M., Kato, S., Kitamura, Y., Hosokawa, H., Davies, J.P., Ioannou, Y.A., Vanier, M.T., et al. (2007). Endosomal accumulation of Toll-like receptor 4 causes constitutive secretion of cytokines and activation of signal transducers and activators of transcription in Niemann-Pick disease type C (NPC) fibroblasts: a potential basis for glial cell activation in the NPC brain. J. Neurosci. *27*, 1879–1891.

Taberner, M., Scott, K.F., Weininger, L., Mackay, C.R., and Rolph, M.S. (2005). Overlapping gene expression profiles in rheumatoid fibroblast-like synoviocytes induced by the proinflammatory cytokines interleukin-1 beta and tumor necrosis factor. Inflamm. Res. *54*, 10–16.

Tada, K., Tanaka, M., Hanayama, R., Miwa, K., Shinohara, A., Iwamatsu, A., and Nagata, S. (2003). Tethering of apoptotic cells to phagocytes through binding of CD47 to Src homology 2 domain-bearing protein tyrosine phosphatase substrate-1. J. Immunol. *171*, 5718–5726.

Tanaka, Y., and Schroit, A.J. (1983). Insertion of fluorescent phosphatidylserine into the plasma membrane of red blood cells. Recognition by autologous macrophages. J. Biol. Chem. *258*, 11335–11343.

Timmer, J.C., and Salvesen, G.S. (2007). Caspase substrates. Cell Death Differ. *14*, 66–72.

Trouw, L.A., Blom, A.M., and Gasque, P. (2008). Role of complement and complement regulators in the removal of apoptotic cells. Mol. Immunol. *45*, 1199–1207.

Truman, L.A., Ogden, C.A., Howie, S.E., and Gregory, C.D. (2004). Macrophage chemotaxis to apoptotic Burkitt's lymphoma cells in vitro: role of CD14 and CD36. Immunobiology *209*, 21–30.

Truman, L.A., Ford, C.A., Pasikowska, M., Pound, J.D., Wilkinson, S.J., Dumitriu, I.E., Melville, L., Melrose, L.A., Ogden, C.A., Nibbs, R., et al. (2008). CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. Blood *112*, 5026–5036.

Tsujimoto, Y., and Shimizu, S. (2005). Another way to die: autophagic programmed cell death. Cell Death Differ. *12* (*Suppl 2*), 1528–1534.

Uematsu, S., and Akira, S. (2007). Toll-like receptors and Type I interferons. J. Biol. Chem. *282*, 15319–15323.

Umetsu, S.E., Lee, W.L., McIntire, J.J., Downey, L., Sanjanwala, B., Akbari, O., Berry, G.J., Nagumo, H., Freeman, G.J., Umetsu, D.T., and DeKruyff, R.H. (2005). TIM-1 induces T cell activation and inhibits the development of peripheral tolerance. Nat. Immunol. *6*, 447–454.

van den Eijnde, S.M., Boshart, L., Baehrecke, E.H., De Zeeuw, C.I., Reutelingsperger, C.P., and Vermeij-Keers, C. (1998). Cell surface exposure of phosphatidylserine during apoptosis is phylogenetically conserved. Apoptosis *3*, 9–16.

Venegas, V., and Zhou, Z. (2007). Two alternative mechanisms that regulate the presentation of apoptotic cell engulfment signal in Caenorhabditis elegans. Mol. Biol. Cell *18*, 3180–3192.

Wang, X., Wu, Y.C., Fadok, V.A., Lee, M.C., Gengyo-Ando, K., Cheng, L.C., Ledwich, D., Hsu, P.K., Chen, J.Y., Chou, B.K., et al. (2003). Cell corpse engulfment mediated by C. elegans phosphatidylserine receptor through CED-5 and CED-12. Science *302*, 1563–1566.

Ward, P.P., Mendoza-Meneses, M., Cunningham, G.A., and Conneely, O.M. (2003). Iron status in mice carrying a targeted disruption of lactoferrin. Mol. Cell. Biol. *23*, 178–185.

Woo, E.-J., Kim, Y.-G., Kim, M.-S., Han, W.-D., Shin, S., Robinson, H., Park, S.-Y., and Oh, B.-H. (2004). Structural mechanism for inactivation and activation of CAD/DFF40 in the apoptotic pathway. Mol. Cell *14*, 531–539.

Wyllie, A.H. (1980). Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature *284*, 555–556.

Xiong, W., Chen, Y., Wang, H., Wang, H., Wu, H., Lu, Q., and Han, D. (2008). Gas6 and the Tyro 3 receptor tyrosine kinase subfamily regulate the phagocytic function of Sertoli cells. Reproduction *135*, 77–87.

Yamaguchi, H., Takagi, J., Miyamae, T., Yokota, S., Fujimoto, T., Nakamura, S., Ohshima, S., Naka, T., and Nagata, S. (2008). Milk fat globule EGF factor 8 in the serum of human patients of systemic lupus erythematosus. J. Leukoc. Biol. *83*, 1300–1307.

Yokota, S., Imagawa, T., Mori, M., Miyamae, T., Aihara, Y., Takei, S., Iwata, N., Umebayashi, H., Murata, T., Miyoshi, M., et al. (2008). Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. Lancet *371*, 998–1006.

Yoshida, H., Kawane, K., Koike, M., Mori, Y., Uchiyama, Y., and Nagata, S. (2005a). Phosphatidylserine-dependent engulfment by macrophages of nuclei from erythroid precursor cells. Nature *437*, 754–758.

Yoshida, H., Okabe, Y., Kawane, K., Fukuyama, H., and Nagata, S. (2005b). Lethal anemia caused by interferon-beta produced in mouse embryos carrying undigested DNA. Nat. Immunol. *6*, 49–56.

Zhang, H.G., Hyde, K., Page, G.P., Brand, J.P., Zhou, J., Yu, S., Allison, D.B., Hsu, H.C., and Mountz, J.D. (2004). Novel tumor necrosis factor alpha-regulated genes in rheumatoid arthritis. Arthritis Rheum. *50*, 420–431.

Züllig, S., Neukomm, L.J., Jovanovic, M., Charette, S.J., Lyssenko, N.N., Halleck, M.S., Reutelingsperger, C.P., Schlegel, R.A., and Hengartner, M.O. (2007). Aminophospholipid translocase TAT-1 promotes phosphatidylserine exposure during C. elegans apoptosis. Curr. Biol. *17*, 994–999.